Kjeldahl method, Kjeldahl Nitrogen Analysis

Danish chemist Johan Kjeldahl (1849–1900) developed what today is well known as the Kjeldahl method for determining nitrogen in organic substances. The original method as presented by Kjeldahl has been continuously improved. These developments have improved environmental and personal safety aspects, increased the speed and versatility of the method, and simplified the entire analytical procedure.

The Kjeldahl method has 3 different steps: digestion, distillation, and titration. Traditionally Kjeldahl flasks with a capacity for 500–800 mL and gas or electric heating have been used for the digestion. For distillation, the addition of water and alkali to the digested sample has been followed by the heating of the flask in order to distill >150 mL distillate. The distilled ammonia has been captured in standardized acid, and back titration with standardized sodium hydroxide has been used. Amethod based on block digestion/steam distillation/boric acid trapping solution having a wide scope of applicability to agricultural products would definitely fulfill a need in the international laboratory community.

Table 1. Results (%) of the comparability studies of the copper/boric acid Kjeldahl method

Sample	Proposed method	AOAC 976.06H[a]	AOAC 990.03[b]
	Nitrogen		
Lysine	14.99	15.266	
Tryptophan	13.58	13.67	
Nicotinic acid	2.37	3.04	
	Crude protein		
Protein block	39.74	39.44	40.53
Meat and bone meal	50.75	48.53	50.86
Albumin	80.73	79.92	79.99
Grass hay	7.3	7.03	7.11
Milk replacer	21.31	21.68	21.21
Birdseed	13.52	13.35	13.2
Dog food	24.62	24.64	24.35
Soybeans	38.42	38.40	38.3
Chinchilla food	18.19	18.14	18.01
Corn silage green chop	7.26	7.17	6.95
Legume hay	18.92	18.91	18.78
Swine pellets	37.32	37.05	37.26
Sunflower seed	16.55	17.05	17.19

Priciple

The material is digested in H2SO4 to convert the protein N to (NH4)2SO4 at a boiling point elevated by the addition of K2SO4 with a Cu catalyst to enhance the reaction rate. Ammonia is liberated by alkaline steam distillation and quantified titrimetrically with standardized acid. Aluminum block heaters increase the efficiency of the digestion.

mixing concentrated alkali with concentrated acid and to prevent the digest from solidifying. Concentrated NaOH is added to neutralize the acid and make the digest basic, and the liberated NH3 is distilled into a boric acid solution and titrated with a stronger standardized acid, HCl, to a colorimetric endpoint. The same endpoint detection system (e.g., indicator, wavelength) must be used for the standardization of the HCl and for the analyte.

The analyte is referred to as “crude” protein because the method determines N, a component of all proteins. In addition, N from sources other than true protein is also determined. (Additional digestion procedures must be used in order to include N from nitrate.) The amount of protein in most materials is calculated by multiplying % N by 6.25, because most proteins contain 16% N.

The H2SO4 and NaOH used are in concentrated form and are highly corrosive. Wear gloves and eye protection while handling the chemicals. Do not mix concentrated acid and NaOH directly. If chemicals are splashed on the skin or in the eyes, flush with copious amounts of water. Seek medical attention. Do not breathe the sulfur oxide fumes produced during digestion.

Apparatus

(a) *Digestion block*.—Aluminum alloy block with adjustable temperature device for measuring and controlling block temperature (Tecator Digestion System 20, 1015 Digestor, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, USA; +1-952-974-9892, Fax: +1-952-974-9823, info@fossnorthamerica.com; or equivalent).

(**b**) *Digestion tubes.*—250 mL.

(**c**) *Distillation units.*—(*1*) *For steam distillation*.—Foss Tecator 2200, or equivalent, to accept 250 mL digestion tubes and 500 mL titration flasks. (*2*) *For steam distillation and autotitration.*—Foss Tecator 2300, or equivalent.

d) *Titration flask*.—500 mL graduated Erlenmeyer flask (for collection and titration of distillate).

(**e**) *Fume exhaust manifold*.—With Teflon ring seals, connected to a water aspirator in a hooded sink.

(**f**) *Weighing paper*.—Low N, Alfie Packers No. 201 (Alfie Packers, Inc., 8901 J St, Ste 10, Omaha, NE 68127, USA), or Fisher 09-898-12A, 3 ×3 in. (76 × 76 mm), or equivalent.

g) *Pipetting dispenser*.—25 mL, adjustable volume, attached to a 5 pint (2.4 L) acid bottle.

Reagents

(**a**) *Sulfuric acid.*—Concentrated, 95–98% H2SO4, reagent grade.

(**b**) *Catalyst*.—7.0 g K2SO4 + 0.8 g CuSO4. (Commercially available in tablet form as 3.5 g K2SO4 and 0.4 g CuSO4 per tablet.)

(**c**) *Sodium hydroxide solution.*—40% (w/w) NaOH, low N (≤5 μg N/g).

(**d**) *Methyl red indicator solution.*—Dissolve 100 mg methyl red in 100 mL methanol.

(**e**) *Bromocresol green indicator solution.*—Dissolve 100 mg bromocresol green in 100 mL methanol.

(**f**) *Boric acid solution.*—4%(w/v). Dissolve 400 gH3BO3 in 5–6 L hot deionized water. Mix and add more hot deionized water to a volume of about 9

L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Adjust to obtain a positive blank of 0.05–0.15 mL with 30 mL H3BO3 solution, using 0.1M NaOH (to increase blank) or 0.1M HCl (to decrease blank). Commercially available.

g) *Boric acid solution.*—1% (w/v). (Optional trapping solution for titrators that automatically begin titration when distillation begins.) Dissolve 100 g H3BO3 in 5–6 L hot deionized water, mix, and add more hot deionized water to a volume of about 9 L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Commercially available.

(**h**) *Hydrochloric acid standard solution.*—0.1000M. Prepare as in **936.15** (*see* A.1.06) or use premade solution of certified specification range 0.0995–0.1005M, and use 0.1000M for calculation. Commercially available.

(**i**) *Reference standards.*—Ammonium sulfate, tryptophan, lysine·HCl, or glycine *p*-toluenesulfonic acid, for use as standard; 99.9%.

(**j**) *Sucrose.*—N-free.

Determination

(a) *Digestion.*—Turn on block digestor and heat to 420°C. Weigh materials, as indicated below, recording each test portion weight (W) to the nearest mg for weights of ≥1 g, and to the nearest 0.1 mg for weights of <1.0 g. Do not exceed 1.2 g. For materials with 3–25% protein, weigh approximately 1.0 g test portion; with 25–50% protein, approximately 0.5 g test portion; and >50% protein, approximately 0.3 g test portion.

(*1*) *Dry feed, forage, cereal, grain, oilseeds*.—Weigh 1 g test portion of ground, well-mixed test portion onto a tared, low N weighing paper. Fold paper around material and drop into a numbered Kjeldahl tube.

2) *Liquid feed.*—Weigh slightly >1 g test portion of well-mixed analytical sample into a small tared beaker. Quantitatively transfer to a numbered Kjeldahl tube with <20 mL deionized water. Alternatively, weigh slightly >1 g well-mixed test portion into a small tared beaker. Transfer to a numbered Kjeldahl tube and reweigh beaker. The differential weight loss corresponds to the amount of test portion actually transferred to the tube.

(b) *Standards.*—Perform quality control analysis and analyses of standards with each batch. The standards available from Hach Co. (PO Box 389, Loveland, CO 80539, USA; +1-800-227-4224 or +1- 970-669-3050), Sigma (St. Louis, MO), J.T. Baker (Phillipsburg, NJ), the National Institute of Standards and Technology (NIST; Gaithersburg, MD) are listed in Table **2001.11C**.

The various ammonium salts and glycine *p*-toluenesulfonate serve primarily as a check on distillation efficiency and accuracy in titration steps because

they are digested very readily. Lysine and nicotinic acid *p*-toluenesulfonate serve as a check on digestion efficiency because they are difficult to digest. Include a reagent blank tube containing a folded low N weighing paper with each batch.

(c) *Digestion.*—Add 2 catalyst tablets to each tube. Add 12 mL H2SO4 to each tube, using pipetting dispenser; add 15 mL for high fat materials (>10% fat). Mixtures may be held overnight at this point. If mixture foams, slowly add 3 mL 30–35% H2O2. Let reaction subside in perchloric acid fume hood or in exhaust system.

Attach heat side shields to tube rack. Place fume manifold tightly on tubes, and turn water aspirator on completely. Place rack of tubes in preheated block. After 10 min, turn water aspirator down until acid fumes are just contained within exhaust hood. A condensation zone should be maintained within the tubes. After bulk of sulfur oxide fumes are produced during initial stages of digestion, reduce vacuum source to prevent loss of H2SO4. Digest additional 50 min. Total digestion time is approximately 60 min.

Turn digestor off. Remove rack of tubes with exhaust still in place, and put in the stand to cool for 10–20 min. Cooling can be increased by using commercial air blower or by placing in hood with hood sash pulled down to increase airflow across tubes. When fuming has stopped, remove manifold, and shut off aspirator. Remove side shields. Let tubes cool. Wearing gloves and eye protection, predilute digests manually before distilling. Carefully add a few milliliters of deionized water to each tube. If spattering occurs, the tubes are too hot. Let cool for a few more minutes. Add water to each tube to a total volume of approximately 80 mL (liquid level should be about half way between the 2 shelves of the tube rack). This is a convenient stopping point.

If digest solidifies, place tube containing diluted digest in block digester, and carefully warm with occasional swirling until salts dissolve. If distilling unit equipped with steam addition for equilibration is used, the manual dilution steps can be omitted. About 70mLdeionized water is then automatically added during the distillation cycle.

(**e**) *Distillation.*—Place 40% NaOH in alkali tank of distillation unit. Adjust volume dispensed to 50 mL. Attach digestion tube containing diluted digest to distillation unit, or use automatic dilution feature, if available. Place graduated 500 mL Erlenmeyer titration flask containing 30 mL H3BO3 solution with indicator on receiving platform, and immerse tube from condenser below surface of H3BO3 solution. (When an automatic titration system is used that begins titration immediately after distillation starts, 1% H3BO3 may be substituted.) Steam distill until ≥150 mL distillate is collected (≥180 mL total volume). Remove receiving flask. Titrate H3BO3 receiving solution with standard 0.1000M HCl to violet endpoint (just before the solution goes back to pink)

Calculations

$$\text{Kjeldahl nitrogen, \%} = \frac{(V_S - V_B) \times M \times 14.01}{W \times 10}$$

$$\text{Crude protein, \%} = \text{\% Kjeldahl N} \times F$$

where VS = volume (mL) of standardized acid used to titrate a test
VB = volume (mL) of standardized acid used to titrate reagent blank
M = molarity of standard HCl; 14.01 = atomic weight of N
W = weight (g) of test portion or standard
10 =factor to convert mg/g to percent
F = factor to convert N to protein.
F factors are 5.70 for wheat, 6.38 for dairy products, and 6.25 for other feed materials.